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Biosynthesis of silver nanoparticles using *Dittrichia graveolens* (Asteraceae) leaves extract: characterisation and assessment of their antioxidant activity

Dittrichia graveolens (Asteraceae) yaprakları kullanılarak gümüş nanopartiküllerin biyosentezi: karakterizasyonu, ve antioksidan aktivitelerinin değerlendirilmesi

Esma Nur Gecer[a], Ramazan Erenler[a*]

[a] Department of Chemistry, Faculty of Arts and Sciences, Tokat Gaziosmanpasa University, 60240, Tokat, Turkey

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**Corresponding author*:
e-mail: rerenler@gmail.com
ORCID: 0000-0002-0505-3190

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ABSTRACT

Nanotechnology has gained great interest recently due to its common applications. Synthesis of silver nanoparticles employing medicinal plants is eco-friendly, low cost, and easy. Silver nanoparticles (i-AgNPs) were synthesized using *Dittrichia graveolens* leaves extract. The structure of i-AgNPs was identified by extensive spectroscopic studies. The antioxidant effect of extract and i-AgNPs was determined by DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and FRAP assays. FTIR spectroscopic study displayed the characteristic vibration of the hydroxyl group at 3262 cm^{-1}. Ultraviolet-Visible (UV-Vis) spectrophotometer exhibited the maximum absorption of i-AgNPs at 451 nm. The particle size of the green synthesized nanostructure was determined as 30.7 nm by scanning electron microscope (SEM). The crystal structure of nanoparticles was established as face-centered cubic (fcc) by X-Ray Diffraction (XRD). DPPH activity of i-AgNPs (13.4±0.34 µg/mL, IC_{50}) was found higher than that of the extract (15.4±0.17 µg/mL, IC_{50}). *Dittrichia graveolens* contains the bioactive compounds that bring out its reducing and stabilizing properties. The extract and i-AgNPs displayed the promising antioxidant effect that may be a raw material for food and pharmaceutical applications.

ÖZ

Nanoteknoloji, yaygın uygulamaları nedeniyle son zamanlarda büyük ilgi görmektedir. Tıbbi bitkiler kullanılarak gümüş nanoparçacıkların sentezi çevre dostu, düşük maliyetli ve kolaydır. *Dittrichia graveolens* yaprakları kullanılarak gümüş nanopartiküller (i-AgNPs) sentezlendi. i-AgNPs yapıları kapsamlı spektroskopik çalışmalarla aydınlatıldı. DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and FRAP yöntemleri kullanılarak, ekstrakt ve i-AgNP'lerin antioksidan etkileri belirlendi. 3262 cm^{-1} de hidroksil grubunun karakteristik titreşimi FTIR spektroskopik çalışma ile belirlendi. UV-Vis spektrometresi, i-AgNPs'lerin maksimum absorpsiyonu 451 nm de gösterdi. Taramalı electron mikroskopu ile (SEM) nanoyapının büyüklüğü 30.7 nm olarak belirlendi. X ışınları kırınımı (XRD) ile nanopartikülün kristal yapısı yüzey merkezli küpik olarak belirlendi. i-AgNPs'in DPPH aktivitesi (13.4±0.34 µg/mL, IC_{50}) ekstrakttan (15.4±0.17 µg/mL, IC_{50}) daha yüksek bulundu. *Dittrichia graveolens*, indirgeyici ve stabilize edici özelliklerini ortaya çıkaran biyoaktif bileşikleri içerir. Ekstrakt ve i-AgNP'ler, gıda ve farmasötik uygulamalar için ham madde olabilecek umut verici antioksidan etki gösterdi.

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1. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field to produce new materials at the nanoscale, which is highly used in science and technology. Nanoparticles (NPs) are accepted as the basic structure of materials based on nanotechnology. The particle size of nanoparticles is considered in the range of 1-100 nm (Akintelu et al., 2020). The nanomaterials have been synthesized using several metals like gold, iron, zinc, copper, and silver. Among them, AgNPs are proved to be the most efficient since silver has significant antimicrobial activity against viruses, bacteria, and other eukaryotic organisms. Moreover, silver nanoparticles have revealed promising potential in many fields and contributed to the progress of nanoscience. Silver nanostructures have attracted great attention last decades, because of their broad applications areas including renewable energy, wastewater treatment, biosensors, pharmaceuticals, clinical equipment, and electronics (Bordoloi et al., 2020).

Physical and chemical processes are applied for silver nanoparticle synthesis. However, they have significant disadvantages compared to green synthesis methods. In green synthesis, no toxic and environmentally harmful chemicals are used to produce nanoparticles. Moreover, they are eco-friendly, low cost, and provide mass production (Burlacu et al., 2019).

In green synthesis, the reduction of Ag^+ to silver metal can be achieved either by bio-species or bioactive compounds obtained from plants or organisms. The bio-based reduction includes viruses, fungi, yeast, bacteria, and microalgae (Lateef et al., 2016). Plants have been utilized for years for medicinal purposes (Akalın et al., 2020; Asad et al., 2020; Eminagaoglu et al., 2020; Palasoglu & Eminagaoglu 2022). Natural products, especially medicinal plants are known as significant starting materials for nanoparticle synthesis owing to their secondary metabolite contents (Erenler et al., 2014; Kaya et al., 2014; Sahin Yaglioglu et al., 2013; Topcu et al., 1999). The bioactive compounds began to be isolated and elucidated from the plants in the 19th century after the development of spectroscopy. Hence, it was understood that the compounds in the plants could show reducing, capping, and stabilization functions for AgNPs synthesis (Erenler & Dag 2021).

Antioxidants are important compounds that inhibit, stabilize, and delay the free radicals including nitric oxide, hydroxyl, superoxide, and peroxyl formed during the oxidative process in the body (Elmastas et al., 2015). The human body has antioxidant enzymes and non-enzymatic compounds to combat oxidative stress (Erenler et al., 2015). In some cases, such as bad living conditions, smoking, ultraviolet radiation, the natural mechanism of antioxidant become insufficient, so, excess free radicals may damage the cell membrane causing ailments such as Alzheimer's, aging, acute toxicity, cardiovascular diseases, diabetes mellitus, rheumatism (Erenler et al., 2017a; Erenler et al., 2016). In addition, they damage to the DNA leading to cancer (Erenler et al., 2017b). Many medicines including antioxidant ingredients have been used effectively for the prevention and treatment of diseases caused by reactive oxygen species. Synthetic and natural antioxidants have been employed efficiently in the food industry. Yet, synthetic antioxidants are limited due to their cancerogenic effect. So, the interest in natural antioxidants has grown steadily for use in medicine, and food (Elmastas et al., 2018; Guzel et al., 2017).

Herein, silver nanoparticles were produced from *Dittrichia graveolens* (L.) Greuter by an eco-friendly, cheap, and easy manner. Antioxidant capacity of extract and i-AgNPs was executed using the DPPH, ABTS, and FRAP assays.

2. MATERIALS AND METHODS

2.1. Chemicals

The chemicals and solvents were supplied commercially from local companies.

2.2. Plant materials

Dittrichia graveolens was obtained from Tokat, Turkey Province, and botanical identification was executed by Dr. Ahmet Ilcim, Department of Biology, Mustafa Kemal University after comparison with the samples deposited at the herbarium, MKUH-1823.

2.3. Synthesis of silver nanoparticles

Dittrichia graveolens leaves (15 g) were collected and dried. The powder material was heated in deionized water (120 mL) for 3 hours at 55 °C. The solid material was filtered and discharged. The deionized water solution of *Dittrichia graveolens* leaves (150 mL) was treated with silver nitrate deionized water solution (0.053 M, 100 mL) for 3 h at 55 °C. The silver nanoparticles were obtained by centrifugation at 10000 rpm for 15 min, then dried by lyophilization (Kumari et al., 2020).

2.4. Characterization of silver nanoparticles

The structure of green synthesized AgNPs was identified by spectroscopic techniques. The maximum absorption of i-AgNPs and antioxidant activity tests were determined by an Ultraviolet-visible (UV-2600) spectrophotometer. Fourier Transform Infra-red (FTIR 4700) spectrometer was employed to determine the functional groups of compounds in the plant extract and stabilized the i-AgNPs. X-ray Diffraction Analysis was executed by a diffractometer (Malvern Panalytical). The morphology of i-AgNPs was established by Scanning Electron Microscope, elemental analysis was presented by an EDAX detector.

2.5. DPPH$^{\bullet}$ free radical scavenging assay

After preparation of stock solutions of extract and i-AgNPs (1 mg/mL), these solution (20, 40, and 80 µL) were completed to 3.0 mL with ethanol. The treatment of 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) solution in ethanol (1.0 mL, 0.26 mM) with each extract and i-AgNPs solution was performed. The solution was vortexed, incubated for 25 min at rt, later absorbance measurement was performed at 517 nm. The activity was determined by the calibration curve of Trolox (Dede et al., 2019).

2.6. ABTS$^{\bullet+}$ radical cation assay

The reaction of 2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (2.0 mM) with sodium persulfate (2.45 mM) at 5 hours yielded the ABTS$^{\bullet+}$ solution. ABTS$^{\bullet+}$ solution was treated with phosphate buffer (pH 7.4, 0.1 mM) and then, the sample (extract and silver nanoparticles) was reacted with ABTS$^{\bullet+}$ at various concentrations. The absorbance measurement was executed at 734 nm (Erenler et al., 2019).

2.7. Ferric reduction antioxidant power (FRAP) assay

Each extract and silver nanoparticles (100 µL, 40-150 µg/mL) was mixed with the phosphate buffer (1.15 mL, 0.20 M, pH 6.7), potassium ferric cyanide (1.0%, 1.25 mL) then, mixture was incubated for 30 minutes at 55°C, then CCl_3COOH (1.25 mL, 10%) and iron (III) chloride ($FeCl_3$) (0.25 mL, 0.1%), were added. After the vortex for 5 min, absorbance measurement was executed on a spectrophotometer at 700 nm (Genc et al., 2019).

2.8. Statistical analysis

Antioxidant activity tests were carried out in triplicate, along with their mean half-maximal inhibitory concentration and SDs. All analyses were executed by GraphPad Prism (version 8.00). The comparisons were executed by one-way analysis of variance (ANOVA) and then Tukey multiple comparison test ($P < 0.05$).

3. RESULTS AND DISCUSSIONS

3.1. UV-Vis spectral analysis of silver nanoparticles

The nanoparticles synthesis was achieved using *Dittrichia graveolens* leaves extract. The absorption peak observed at 451 nm revealed the development of i-AgNPs. The color change from yellow (1) to dark brown (2) proved the desired structure as well. Ag^{+} ions were reduced to silver metal by the natural compounds found in *Dittrichia graveolens* leaves extract. The formation of nanostructures included three stages, reduction, clustering, and nanoparticles growth (Figure 1).

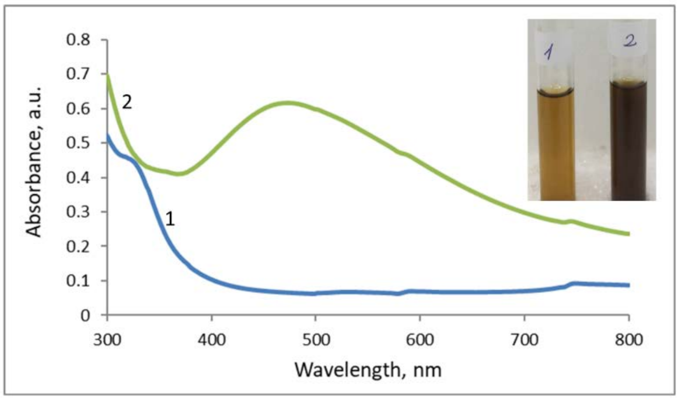

Figure 1. Uv-Vis spectrum of extract (1) and i-AgNPs (2). Aqueous solution of extract (1) and i-AgNPs (2)

3.2. FTIR analysis

FTIR spectroscopy revealed the functional groups of plant secondary metabolites which were reliable for the reduction of silver ions as well as stabilization of nanostructures. The slight change in the spectral values of the extract and i-AgNPs confirmed the proposed structures (Figure 2). While the reduction of silver ions, some functional groups of the compounds in the extract were oxidized. In the FTIR spectrum, observation of broad signal at 3262 cm^{-1} belonged to the hydroxyl. The peak that appeared at 2931 could be attributed to the C-H stretching of alkane. The absorption signal at 1594 could be due to the N-H bending. The peak that appeared at 1392 cm^{-1} belonged to the O-H bending. The peak observed at 1259 cm^{-1} could be due to the C-O stretching. The strong peak at 1017 cm^{-1} could be attributed to the C=C bending vibration signal.

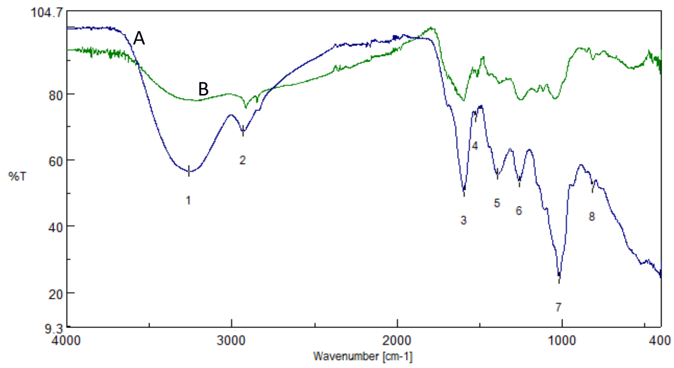

Figure 2. FTIR spectrum of extract (A) and i-AgNPs (B). **1:** 3262 cm^{-1}, **2:** 2931 cm^{-1}, **3:** 1594 cm^{-1}, **4:** 1521 cm^{-1}, **5:** 1392 cm^{-1}, **6:** 1259 cm^{-1}, **7:** 1017 cm^{-1}, **8:** 813 cm^{-1}

3.3. X-ray diffraction

X-ray diffraction pattern presented the crystal nature of silver nanoparticles synthesized from *Dittrichia graveolens* leaves extract. The face-centered cubic crystal structure of i-AgNPs was proved by the diffraction peak (2θ) at the angle of 38.1°, 44.3°, 64.4°, and 77.4° indexed in the plane (111), (200), (220), and (311) respectively. The crystal size of i-AgNPs was calculated by the Debye-Scherrer formula (1)

$D= 0.9\ \lambda/\beta \cos\theta$ (1)

D represents the nanostructure diameter, wavelength of the radiation was signified by λ, the half-maximum value of XRD diffraction was symbolized by β, θ is the half diffraction angle. The particle size was calculated as 30.7 nm (Figure 3).

3.4. Scanning Electron Microscope

SEM image presented the morphology of nanoparticles structure (Figure 4). The dispersion of agglomerated clusters distributed over the surface was displayed by SEM image. The energy dispersive analysis (EDX) also confirmed the formation of the nanostructure. Moreover, the strong peak of Ag in the EDX spectrum at around 3 and 3.3 keV proved the desired structure (Figure 5). Elemental analysis results showed the silver nanoparticle formation as 61.96% (Figure 5).

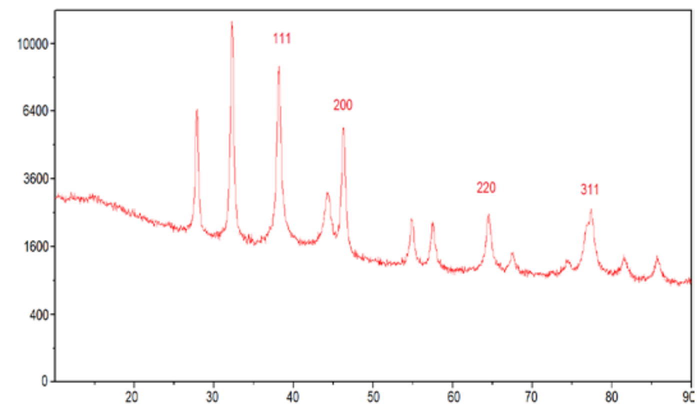

Figure 3. XRD pattern of i-AgNPs

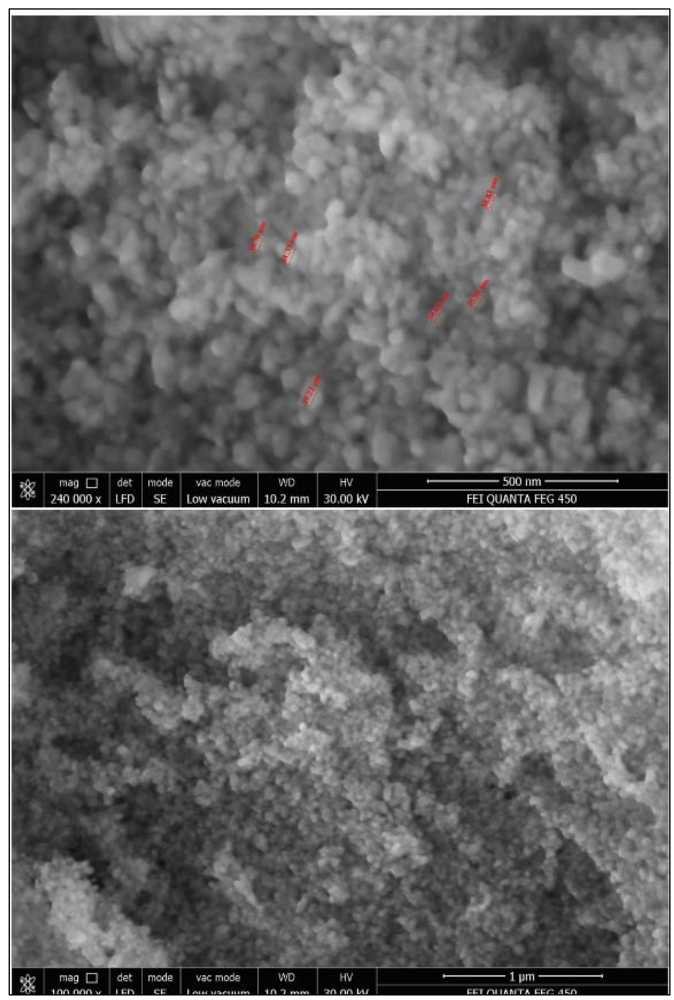

Figure 4. SEM image of i-AgNPs

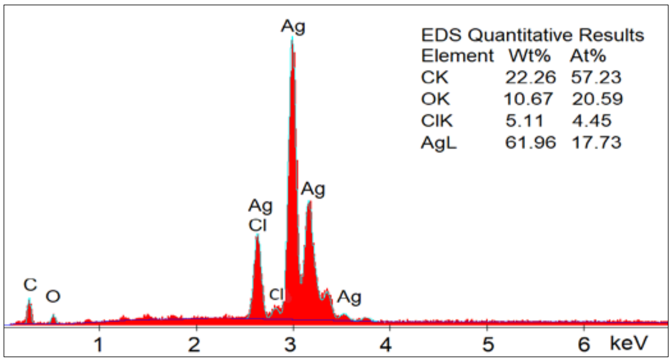

Figure 5. EDX spectrum and elemental analysis of i-AgNPs

3.5. Antioxidant activity

The antioxidant activity of extract and nanoparticles was investigated using the DPPH, ABTS, and FRAP assays (Figure 6). In the DPPH test, it was observed that i-AgNPs (13.4±0.34 µg/mL, IC_{50}) has significantly higher activity than that of the extract (15.4±0.17 µg/mL, IC_{50}). Regarding ABTS test, the extract activity (4.8±0.05 µg/mL, IC_{50}) was found higher than i-AgNPs (6.8±0.04 µg/mL, IC_{50}). Both extract and i-AgNPs displayed better activity than standard BHT (8.3±0.1 µg/mL, IC_{50}). In reducing power activity results, extract and i-AgNPs revealed the same activity. However, their activity was detected lower than standards, BHA and BHT. Consequently, i-AgNPs and extract obtained from *Dittrichia graveolens* are promising antioxidant agents. This study is consistent with the previous study. AgNPs were synthesized using *Syzygium cumini* fruit extract and they revealed considerable antioxidant activity (Mittal et al., 2014). In addition, *Chenopodium murale* leaf extract was used for the synthesis of AgNPs and it was reported that the corresponding nanostructures displayed significant antioxidant activity (Abdel-Aziz et al., 2014). Nanoparticles were synthesized and characterized using the *Echinacea purpurea* leaves extract and their significant antioxidant activities were determined (Gecer et al., 2021). Besides the plant extracts, pure compounds have been used for the synthesis of silver nanoparticles as well. The synthesis of silver nanoparticles was achieved using oleuropein isolated from olive leaves that exhibited substantial antioxidant effects (Genc et al., 2020). The nanoparticles synthesized from *Dittrichia graveolens* can be a promising material for the antioxidant agents as well as a drug material for the diseases caused by reactive oxygen species. The nanoparticles were also reported to possess considerable anticancer activity (Karan et al., 2022).

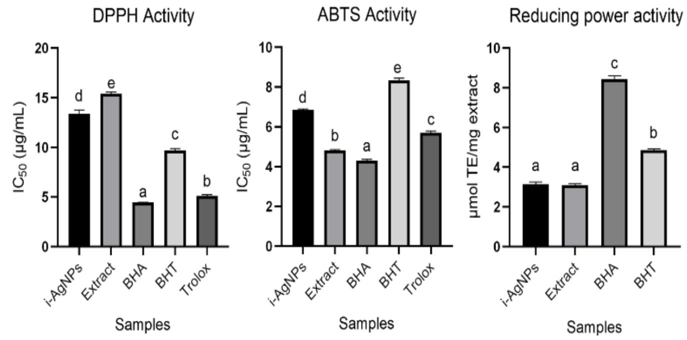

Figure 6. Antioxidant activity of extract and silver nanostructures

4. CONCLUSION

A simple, eco-friendly, rapid, low-cost approach was developed using *Dittrichia graveolens* leaves extract for nanoparticles synthesis. This is the bio-reduction process that takes place by the bioactive compounds found in the plants. The bioactive compounds in the extract acted both as reducing and capping agents. The structure of green synthesized silver nanoparticles was elucidated by spectroscopic techniques such as UV-Vis, FTIR, XRD, SEM, and EDX. The synthesized nanoparticles were spherical with an average size of 30.7 nm. The aggregation was observed in the various part of the SEM image. Due to their promising antioxidant effect, nanoparticles have the potential to be used in pharmaceutical products that can be employed against different diseases caused by reactive oxygen species.

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